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IN VITRO ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS ON GROWTH OF PHYTOPATHOGENIC FUNGI

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SUMMARY

Eleven essential oils (clove, rosemary, cinnamon leaf, sage, scots pine, neroli, peppermint, aniseed, caraway, lavander, common thyme) were tested for in vitro antifungal activity on twelve plant pathogenic fungi (Fusarium graminearum, F. verticillioides, F. subglutinans, F. oxysporum, F. avenaceum, Diaporthe helianthi, Diaporthe phaseolorum var. caulivora, Phomopsis longicolla, P. viticola, Helminthosporium sativum, Colletotrichum coccodes, Thanatephorus cucumeris). The results indicated that all oils except scots pine and neroli had antifungal activity against some or all tested fungi. The best antifungal activity had common thyme, cinnamon leaf, clove and aniseed oils. When compared to control, scots pine, neroli and sage oils stimulated mycelium growth of some investigated fungi.

Key-words: essential oil, inhibition, plant disease, mycelial growth

INTRODUCTION

More than 1300 plant species are known to be potential sources of antimicrobial components but only some of them have been studied scientifically (Wilkins and Board, 1989, Paster et al., 1990.). For instance, some previous studies evaluated the inhibitory activity of essential oils on fungi. Cardwell and Dongo (1994) tested extracts from nine plant species on mycelial growth of *Apergillus flavus* L.; Manohar et al. (2001) researched origano commercial oil against *Candida albicans* (Robin) Berkhout; Marin et al. (2004) tested cinnamon, clove, oregano, palmarosa and lemongrass oils against *Fusarium graminearum* Schw., Hadizadeh et al. (2009) analyzed antifungal potential of five essential oils against *Alternaria alternata* (Fr.) Keissl. Burgiel and Smaglowski (2008) described complete growth inhibition of *Fusarium culmorum* (W.G. Smith) Sacc. and *B. cinerea* on media with a 0.5% addition of tea tree oil. In addition, some studies reported positive effect of essential oils in reducing mycotoxin accumulation in maize grain (Marin et al. 2004, Velluti et al. 2004).

The aim of this research was to test in vitro the effect of 11 commercial essential oils on mycelial growth of 12 plant pathogenic fungi.

MATERIAL AND METHODS

The effect of clove (*Eugenia caryophyllus* (Sprengel) Bullock & Harr.), rosemary (*Rosmarinus officinalis* L.), cinnamon leaf (*Cinnamomum verum* Presl.), sage (*Salvia officinalis* L.), scots pine (*Pinus sylvestris* L.), neroli (*Citrus aurantium* L. *ssp. amara* Engl.), peppermint (*Mentha piperita* L.), aniseed (*Pimpinella anisum* L.), caraway (*Carum carvi* L.), lavander (*Lavandula angustifolia* Mill. *ssp. angustifolia*) and common thyme (*Thymus vulgaris* L.) oils on growth of *F. graminearum*, *Fusarium verticillioides* (Sacc.) Nirenberg, *Fusarium subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas, *Fusarium oxysporum* Schlecht. emend Snyder and Hansen, *Fusarium avenaceum* Fr. Sacc., *Diaporthe helianthi* Munt.-Cvet. et al., *Diaporthe phaseolorum var. caulivora* (Lehman) Wehmeyer, *Phomopsis longicolla* Hobbs., *Phomopsis viticola* (Sacc.) Sacc., *Helminthosporium sativum* Pamm., King and Bakke, *Colletotrichum coccodes* (Wallr.) Hughes and *Thanatephorus cucumeris* (Frank) Donk was evaluated. Fungi were isolated from wheat grains (*F. graminearum, F. avenaceum, H. sati-*

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vum), maize stalks (*F. verticillioides*, *F. subglutinans, F. oxysporum),* sunflower stalks (*D. helianthi*), soybean stalks (*D. phaseolorum var. caulivora*), soybean grains (*P. longicolla*), grape canes (*P. viticola*), common bean roots (*T. cucumeris*) and *Abutilon theophrasti* Med. roots (*C. coccodes*). The essential oils were produced in Pranarom International (Belgium) and analysed in Pranarom laboratory by GC-MC (gas chromatography-mass spectrometry). Antifungal assays were carried out in vitro according to Saikia et al. (2001). A 5-mm diameter sterilized filter paper disc was placed in the center of Petri dish (9 cm diameter) with PDA and loaded with 5 μl of essential oil. Four discs (5 mm diameter) of mycelial plugs were cross placed into each Petri dish. Petri dishes were kept in thermostat at 22ºC and for 12/12 h light/dark regime. The inhibition zones around the paper disc were measured after eight days as the distance from the margine of the colony to the filter paper. The experiment was performed in four replications. Research results were analyzed statistically by ANOVA and LSD using Statistica for Windows version 9.

RESULTS AND DISCUSSION

The inhibitory effect of twelve essential oils against some economically important plant pathogenic fungi are shown in Table 1. Our research showed that all investigated oils except scots pine and neroli had antifungal activity against some or all researched fungi. The highest degree of antifungal activity showed common thyme oil followed by cinnamon leaf oil (Photo 1), clove oil and aniseed oil.

Table 1. Inhibition zone (mm) eight days after inoculation

Tablica 1. Zona inhibicije (mm) osam dana nakon inokulacije

	DPC	PV	DH	PL	HS	CC	TC	FO	FA	FG	FS	FV
Clove/ *klinčićevac*	23.06	27.13	26.38	20.75	21.63	23.25	0	16.70	8.50	0	7.81	10.50
Rosemary/ *ružmarin*	0.56	7.50	10.13	0	12.25	9.75	0	0	0	0	1.19	0
Cinnamon leaf / *list cimeta*	18.56	32.31	28.06	22.82	18.38	22.25	0	16.80	8.80	0	7.00	8.50
Sage/ *kadulja*	1.75	8.88	2.94	0	4.44	11.90	0	0	0	0	0.88	4.44
Scots pine/ *bor*	0.63	10.50	4.06	0	3.94	8.31	0	0	0	0	1.13	0
Neroli/ *gorka naranča*	1.06	7.31	4.94	0.56	17.25	8.19	0	0	0	0	0.50	0
Peppermint/ *metvica*	1.63	18.75	11.31	0	9.50	12.75	0	9.80	0	3.25	4.00	8.06
Aniseed/ *anis*	6.06	17.69	16.75	11.69	19.38	21.44	2.63	17.20	25.00	0	8.88	8.63
Caraway/ *kim*	1.00	17.75	17.69	0.38	17.50	14.38	0	4.70	0	0	6.31	3.69
Lavander/ *lavanda*	1.63	14.50	10.31	1.31	3.56	10.94	0	0	0	0	0.81	1.81
Common thyme/ *timijan*	35.00	35.00	35.00	34.38	29.31	31.50	0	24.50	16.00	24.38	26.00	24.38
Control/ *kontrola*	0.31	8.25	10.88	0	0	1.50	0	0	0	0	8.06	0
LSD 0.05 0.01	2.50 3.29	3.51 4.61	3.48 4.57	2.02 2.65	1.09 1.43	2.22 1.43	1.73 2.28	1.90 2.60	1.10 1.40	3.44 4.52	3.13 4.12	1.10 1.45

DPC - *D. p. var. caulivora*; PV – *P. viticola*; DH – *D. helianthi*; PL – *P. longicolla*; HS – *H. sativum*;
CC – *C. coccodes*; TC – *T. cucumeris*; FO – *F. oxysporum*; FA – *F. avenaceum*; FG – *F. graminearum*; FS – *F. subglutinans*; FV - *F. verticillioides*

They had a statistically significant negative impact on mycelium growth of all investigated fungi except *T. cucumeris* and *F. graminearum*.

Common thyme essential oil showed complete inhibition on mycelial growth of *D.p. var. caulivora*, *D. helianthi*, *P. viticola*, 98.23% inhibition on mycelial growth of *P. longicolla* and 90% inhibition on mycelial growth of *C. coccodes*. Those results are in accordance with the strong toxic properties of thyme oil and its active compounds, such as thymol and carvacrol, against a large number of microorganisms described by Soliman and Badea (2002). The major compounds found in the thyme oil used in our research were thymol (38.61%) and p-cymene (25.02%). In our research cinnamon leaf oil showed 53.02, 80.17 and 92.31% inhibition on mycelial growth of *D.p. var. caulivora*, *P. viticola* and *D. helianthi*, respectively. The major compound of our cinnamon leaf oil was eugenol (75.43%) having, according to Wang et al. (2010), strong inhibitory effect against *Botrytis cinerea* (De Bary) Whetzel and *Sclerotinia sclerotiorum* (Lib.) De Bary. Edris and Ferrag (2003) indicated synergistic effect of different compounds present in natural essential oils. Bartyńska and Budzikur-Ramza (2001) described high toxicity of eucalyptus, lavender and rosemary oils against *Fusarium* spp. On the contrary, in our research lavander (linalol 37.04%) and rosemary (1-8 cineole 44.40%) oils did not have inhibitory effect on

mycelium growth of the investigated *Fusarium* species. Only thyme oil showed statistically significant inhibition on mycelial growth of all researched *Fusarium* species.

On the other hand, only aniseed oil (anethole 83.50%) had statistically significant negative impact on mycelium growth of *T. cucumeris* and peppermint (menthol 41.06%) and common thyme oils on mycelium growth of *F. graminearum*.

When compared to control, scots pine, neroli and sage oils stimulated mycelium growth of *D. helianthi*, scots pine, sage, peppermint and lavander oils stimulated mycelium growth of *H. sativum* (Photo 2) while rosemary oil stimulated mycelium growth of *F. subglutinans*.

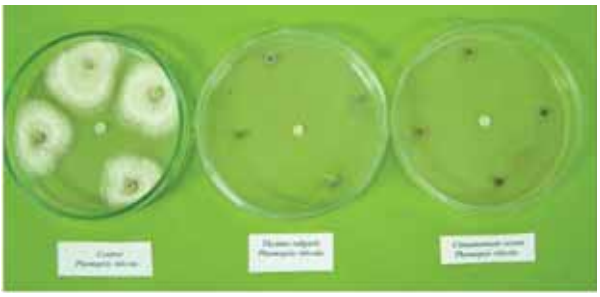

Photo 1. Influence of common thyme and cinnamon leaf essential oils on mycelium growth of *P. viticola*

Slika 1. Utjecaj eteričnog ulja timijana i cimeta na porast micelija P. viticola

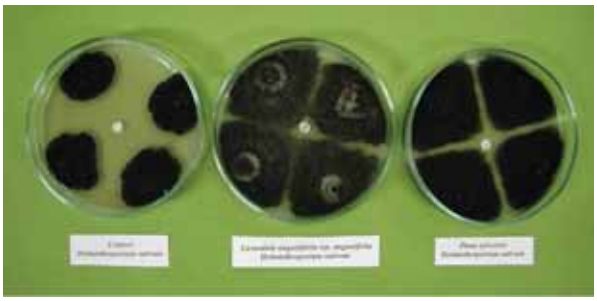

Photo 2. Influence of lavander and scots pine essential oils on mycelium growth of *H. sativum*

Slika 2. Utjecaj eteričnog ulja lavande i bora na porast micelija H. sativum

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ANTIFUNGALNO DJELOVANJE ETERIČNIH ULJA NA PORAST FITOPATOGENIH GLJIVA IN VITRO

SAŽETAK

U radu je testiran utjecaj jedanaest vrsta eteričnih ulja (klinčićevac, ružmarin, list cimeta, kadulja, bor, gorka naranča, metvica, anis, kim, lavanda, timijan) na porast micelija dvanaest fitopatogenih gljiva (Fusarium graminearum, F. verticillioides, F. subglutinans, F. oxysporum, F. avenaceum, Diaporthe helianthi, Diaporthe phaseolorum var. caulivora, Phomopsis longicolla, P. viticola, Helminthosporium sativum, Colletotrichum coccodes, Thanatephorus cucumeris). Sva eterična ulja koja smo koristili, izuzev ulja bora i gorke naranče, pokazala su određeno inhibitorno djelovanje prema nekim ili svim istraživanim gljivama. Najbolje antifungalno djelovanje imala su ulja timijana, lista cimeta, klinčićevca i anisa. U usporedbi s kontrolom, ulja bora, gorke naranče i kadulje pozitivno su utjecala na porast micelija nekih gljiva.

Ključne riječi: eterična ulja, inhibicija, biljna bolest, porast micelija

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